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(54) Apparatus and process for the synthesis of chemical compounds

(57) An improved apparatus for the synthesis of more complex oligonucleotide compounds from simpler chemical compounds wherein at least one of said simpler chemical compounds is carried on a solid matrix comprising a plurality of beads located within a reaction chamber and is sequentially subjected to a plurality of other simpler chemical compounds passed through the chamber in a stream, causing chemical interaction between the simpler chemical compounds to form said more complex compounds.

A novel process which comprises the introduction into a primary reaction zone containing therein a solid support, a plurality of fully activated nucleosides and permitting said plurality of nucleosides to react simultaneously on said solid support to form an oligonucleoside.

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BB3

FIG. 1A

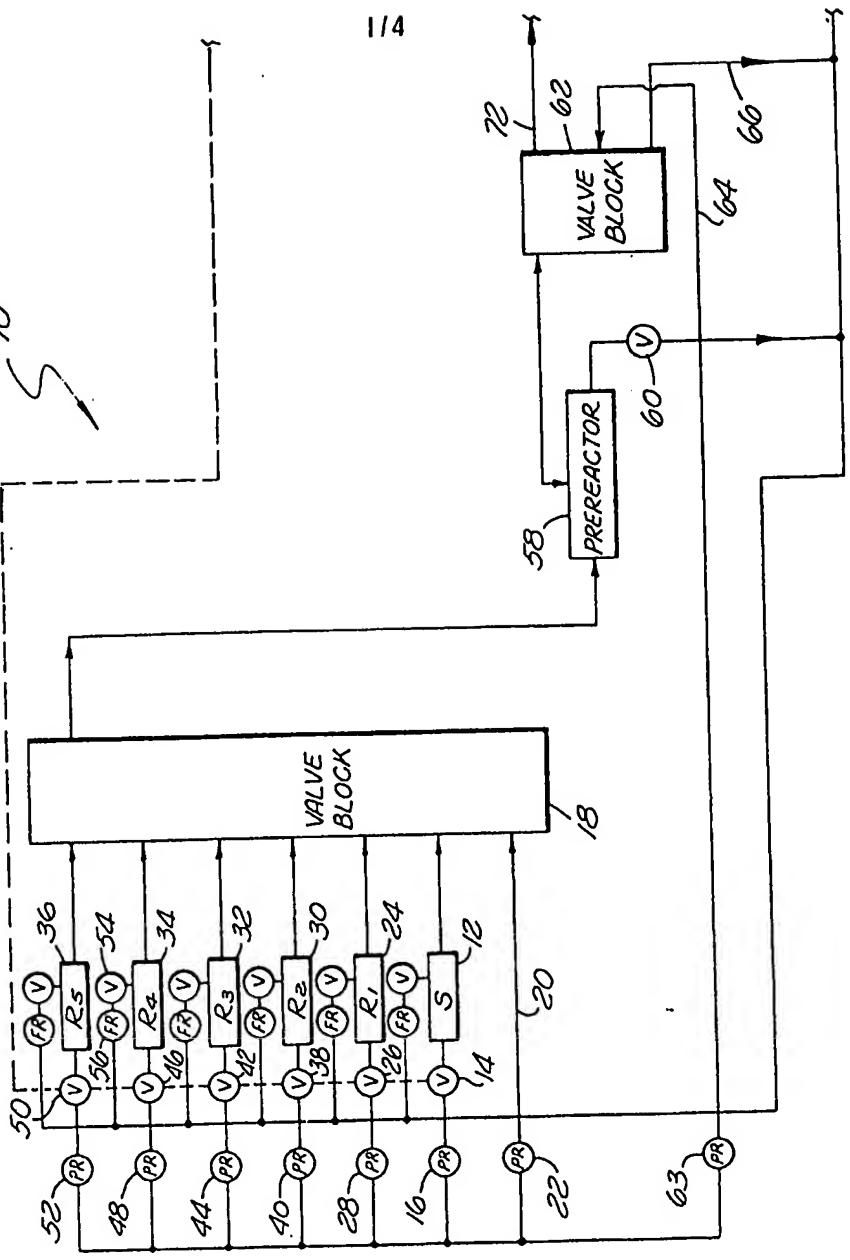
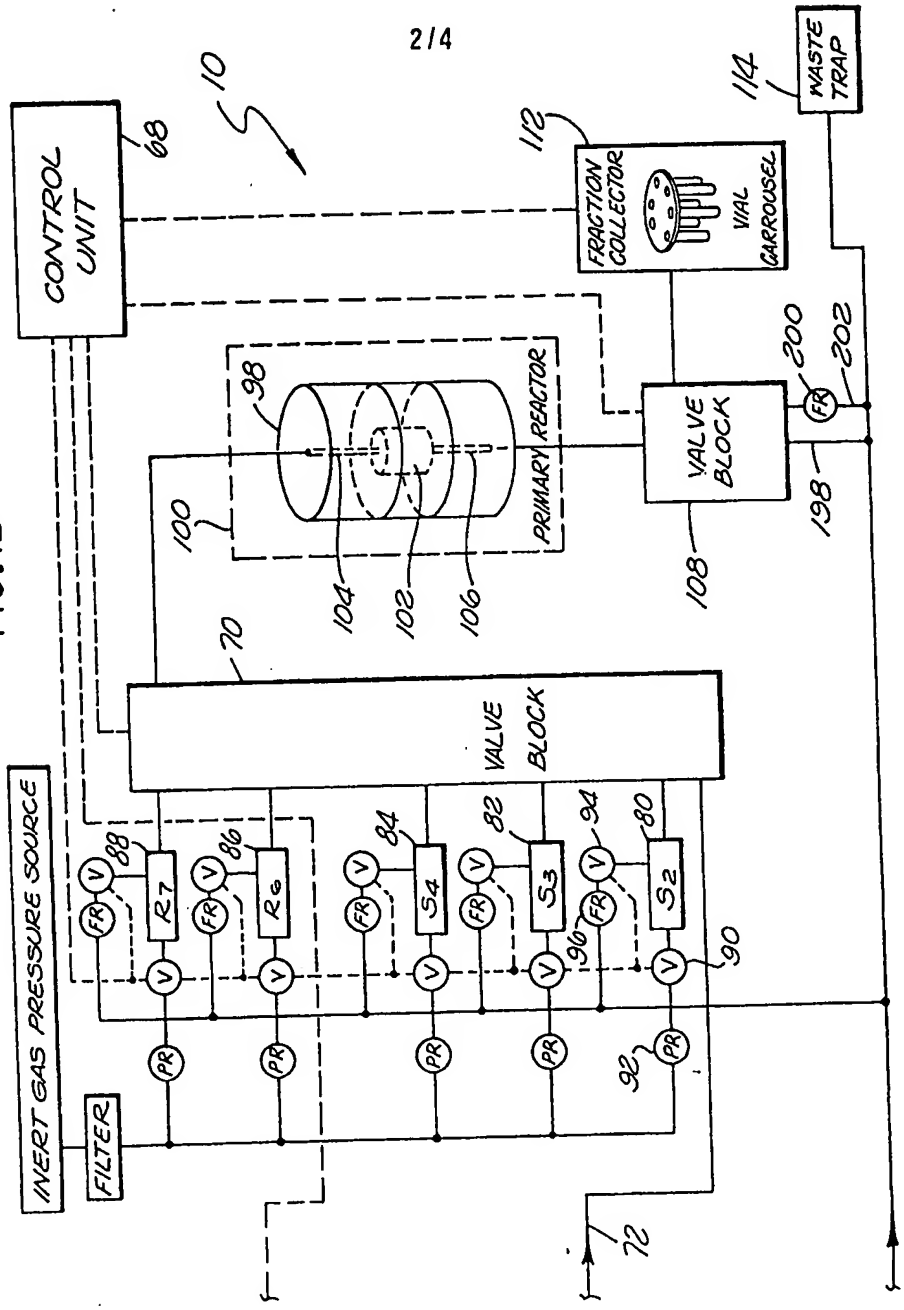

10
VALVE BLOCK
18
PREREACTOR
58
VALVE BLOCK
72
62
60
64
66
R5
R4
R3
R2
R1
S
36
54
34
32
30
24
12
56
46
42
38
26
14
50
20
52
48
44
40
28
16
22
63

FIG. 1B


CONTROL UNIT
68
10
FRACTION COLLECTOR
VIAL CARROUSEL
112
WASTE TRAP
114
VALVE BLOCK
200
202
198
108
PRIMARY REACTOR
98
100
104
102
106
70
VALVE BLOCK
INERT GAS PRESSURE SOURCE
FILTER
88
86
84
82
94
80
R7
R6
S4
S3
S2
96
90
92
72

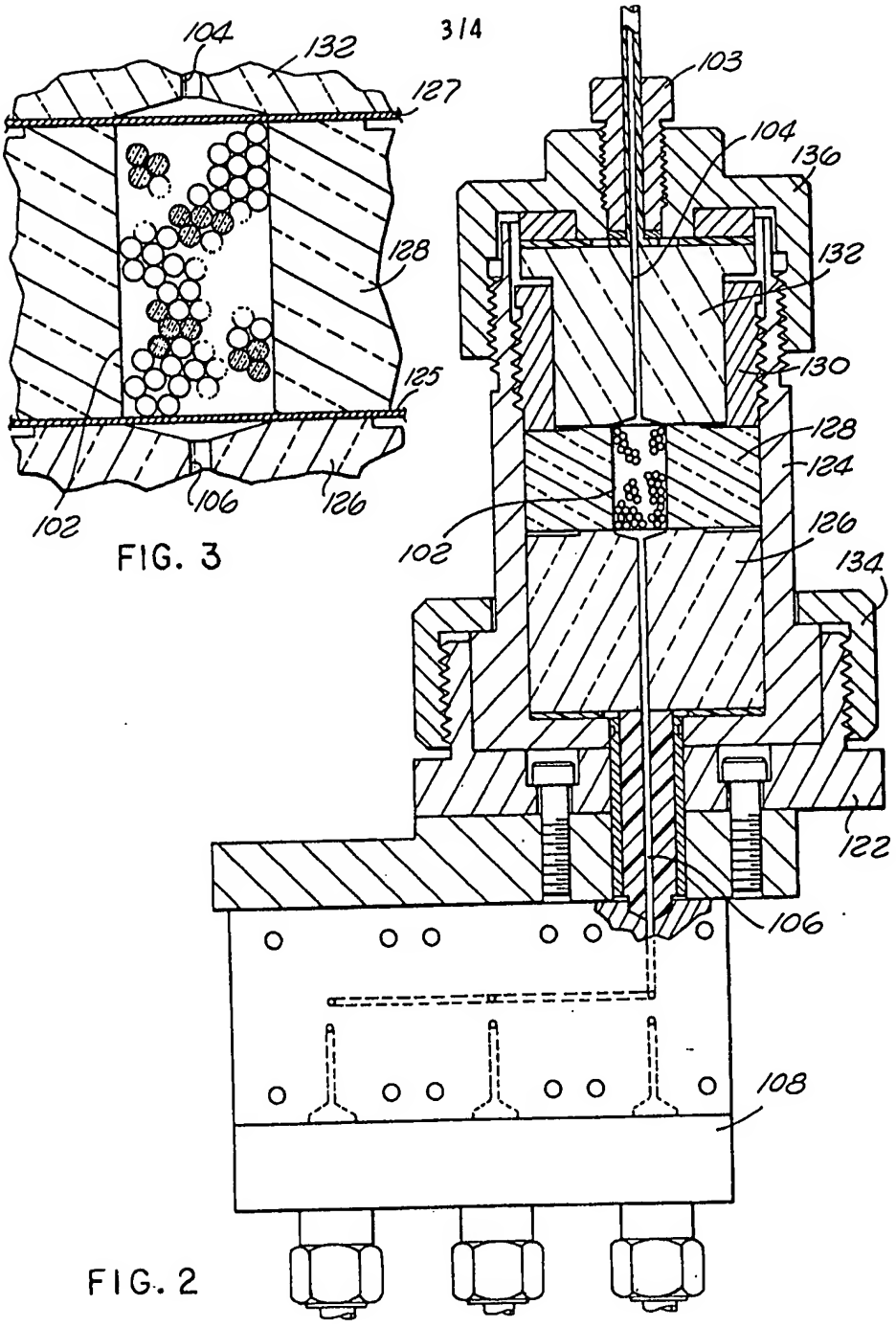

314
104
132
127
128
125
102
106
126
103
104
136
132
130
128
124
126
134
122
106
108

FIG. 3

FIG. 2

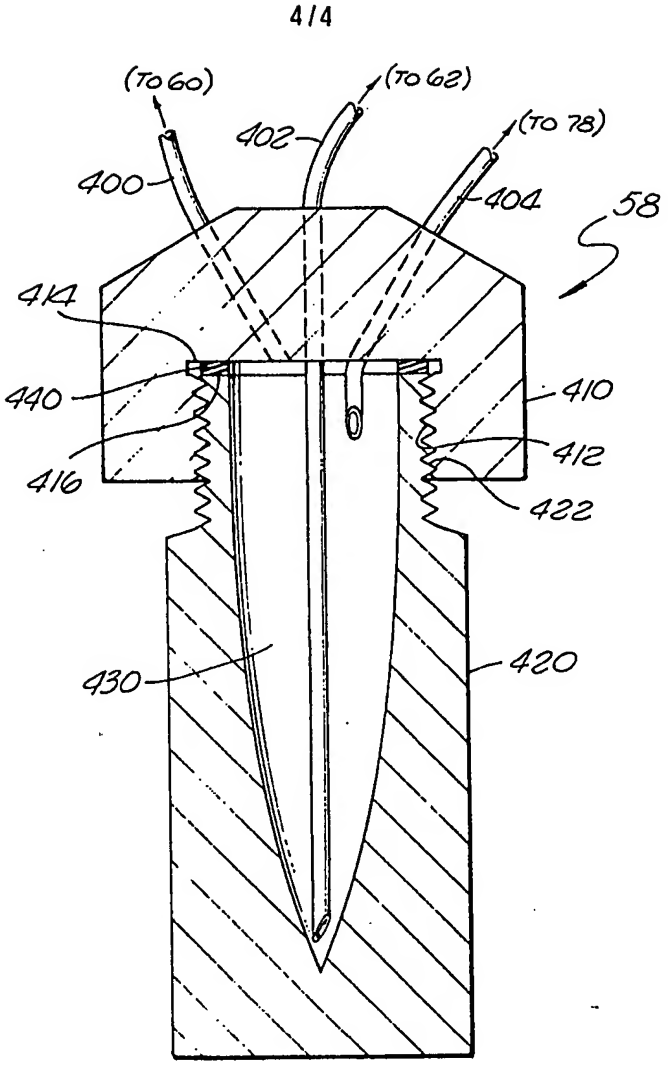

414
(TO 60)
(TO 62)
(TO 78)
402
400
404
58
414
440
410
412
416
422
430
420

FIG. 4

SPECIFICATION

Apparatus and process for the synthesis of chemical compounds

The emergence of recombinant DNA (deoxyribonucleic acid) and genetic engineering as important methodologies for both basic and applied research has been made possible by development of several experimental techniques that, when used together, enable far more complex manipulation of genetic material and living cells than was dreamed possible less than ten years ago. These techniques allow dissection of the human genome into individual genes that can be transferred into microorganisms such as bacteria. There, the genes can be reproduced by the bacteria in such quantities as to allow their chemical structure to be defined. Moreover, the microorganisms containing the human gene can be induced to produce large quantities of the protein product encoded by the gene, thus permitting production of large amounts of scarce human proteins such as interferon, growth hormone, and insulin.

One of the experimental techniques that has played a key part in the rapid development of genetic engineering is the chemical synthesis of oligonucleotides of definedstructure. Such oligonucleotides are often an essential part of the process of isolating a single gene from a library containing tens of thousands to millions of genes that may be expressed by an individual living cell at any one time. Because of the unique chemical structure of DNA, a piece of DNA with a particular sequence will recognize and bind to only another piece with a complementary sequence, ignoring all other pieces of DNA in a complex mixture with the wrong sequences. Therefore, small pieces of chemically synthesized DNA can be used as unique molecular probes to locate much larger pieces of DNA, even entire genes, that contain the appropriate complementary sequence. The correct sequence to incorporate into the probes can be deduced from the structure of the protein encoded by the gene by translating, using the genetic code, protein sequence into DNA sequence. Since the genetic code is degenerate, i.e. most chemical units in the protein sequence can be encoded by several groups of chemical units in the DNA sequence, often a set of DNA probes containing related but nonidentical sequences must be synthesized in order to insure that the one sequence complementary to the desired gene is made. This places severe constraints on the methods used to synthesize DNA. In order for them to be routinely useful, they must be rapid, simple, reliable, and inexpensive, because often several dozen different DNA molecules must be synthesized for locating a single gene.

A process for synthesizing oligonucleotides has been developed by Caruthers et al.(M. D. Matteucci and M. H. Caruthers, J. Amer. Chem. Soc. 103, 3185-3191, 1981; S. L. Beaucage and M. H. Caruthers, Tet. Lett. 22, 1859-1862, 1981). These oligonucleotides are synthesized by the stepwise addition of monodeoxynucleosides to a deoxynucleoside covalently attached to a silica gel polymer support. Individual steps for each addition involve condensation of the activated nucleoside as a phosphite ester (step 1), covalent blockage of unreacted nucleoside 5′-hydroxyl groups (step 2), oxidation of the phosphite to the phosphate (step 3), and removal of the 5′-blocking group from the nucleoside added in each cycle (step 4). Once the desired number of monomers have been added to the growing chain, the completed oligonucleotide is removed from the silica support and the remaining blocking groups are removed from the oligonucleotide by treatment with thiophenol and ammonium hydroxide.

The process of Caruthers et al. has the following features:

(1) The oligonucleotides are synthesized on an insoluble polymer support. Since multistep syntheses are involved, this development simplifies the purification of synthetic intermediates. Excess reagents and most unwanted reaction by-products are washed from the support by solvents without removing the growing polymer.

(2) Activated nucleoside phosphites are the intermediates used for synthesis of oligonucleotides. These phosphites react rapidly and in high yield. These are ideal characteristics for cyclical condensation reactions being completed on a polymer support.

(3) A series of reagents were developed by Caruthers et al. ffor the selective removal of 5′-blocking groups from nucleosides, nucleotides, and oligonucleotides. These reagents are Lewis acids such as $ZnBr_2$, BF_3, $AlCl_3$, and $TiCl_4$. These reagents effectively remove ether-protecting groups without degradation of preformed oligonucleotides.

(4) The total synthetic process is extremely rapid. Each monodeoxynucleoside addition including all processing steps requires about 2-1/2 hours.

(5) Yields exceeding 95% are observed with each of the activated mononucleosides.

The polymer support used in the Caruthers et al. process is composed of one of four appropriately-blocked nucleosides joined covalently to macroporous silica gel beads.

The initial step in the Caruthers et al. process is the synthesis of activated nucleosides. All four nucleosides contain a 5′-blocking group such as di-p-anisylphenylmethyl (dimethoxytrityl, DMTr) ether. However, any other suitable 5′-protecting group can be used. It is also desirable to protect the amino groups on cytosine, adenine, and guanine. Benzoyl, isobutyryl, or similar groups can be used. There-

fore, 5'-O-dimethoxytrityl-deoxythymidine (DMTrd(T)), 5'-O-dimethoxytrityl-N-benzoyldeoxycytidine (DMTrd(bzC)), 5'-O-dimethoxytrityl-N-benzoyldeoxyadenosine (DMTrd(bzA)), and 5'-O-dimethoxytrityl-Nisobutyryldeoxyguanosine (DMTrd(ibG)) have been used as protected nucleosides.

The protected nucleosides must be activated for coupling. Ideally, the activated nucleoside must be stable and easy to handle and, at the same time, must be highly reactive toward the unprotected 5'-hydroxyl group of the nucleoside to which it will couple. These requirements are best met by synthesis of a stable, partially-activated phosphite group that can be converted in situ to the desired reactive compound in a simple reaction. Such compounds, described by Caruthers et al include the nucleoside phosphoramidites. These may be synthesized by the following procedure. DMTr-nucleoside (1 mmol) is dissolved in 3 ml of dry, acid-free chloroform and diisopropylethylamine (4 mmol) in a 10 ml reaction vessel preflushed with dry nitrogen. Chloro-N,N-dialkylaminomethoxyphosphine (2 mmol, the alkyl groups may be methyl, ethyl, isopropyl, or similar groups) is added dropwise (30-60 sec) by syringe to the solution under nitrogen at room temperature. After 15 min., the solution is transferred with 35 ml of ethyl acetate into a 125 ml separatory funnel. The solution is extracted four times with an aqueous, saturated solution of sodium chloride (80 ml). The organic phase is dried over anhydrous sodium sulfate and evaporated to a foam under reduced pressure. The foam is dissolved with toluene (10 ml) or ethyl acetate (10 ml) and the solution is added dropwise to 50 ml of cold hexanes ($-78°C$) with vigorous stirring. The cold suspension is filtered and the white powder is washed with 75 ml of cold hexanes ($-78°C$). The white powder is dried under reduced pressure and stored under nitrogen.

In order to begin the synthesis of the desired oligonucleotide, the first monomer must be coupled to a solid support, typically a high-performance liquid chromatography grade silica gel or controlled-pore glass, both macroporous supports with large surface areas. The support is first derivatized to permit attachment of the monomer. Caruthers et al. have described a suitable procedure that involves reaction of the silica with (3-aminopropyl) triethoxysilane, reaction of the amino-derivatized silica with succinic anhydride, and blockage of any remaining silanol groups with trimethylsilyl chloride. Then the desired DMTr-nucleoside is coupled to the carboxyl group attached to the silica by using dicyclohexylcarbodiimide in anhydrous pyridine. Residual carboxyl groups remaining after 40-hr. treatment are blocked by treatment first with p-nitrophenol and then morpholine. The resulting product contains approximately 50 micromoles of nucleoside per gram of silica.

The steps involved in attaching the next and subsequent nucleosides are summarized as follows.

First, the 5 -DMTr group is removed from the attached nucleoside by treatment with acid (typically $ZnBr_2$ in nitromethane or trichloroacetic acide in chloroform). Next, the silica is washed with solvents to remove the cleavage reagent. These solvents include, for $ZnBr_2$, n-butanol and 2,6-lutidine in tetrahydrofuran followed by tetrahydrofuran, and, for trichloroacetic acid, nitromethane followed by methanol followed by acetonitrile. Then, the desired phosphoramidite is activated by treatment with 10-20 molar equivalents of tetrazole and added to the silica gel to couple to the free 5'-hydroxyl group of the nucleoside it contains. Both the activated nucleoside and the tetrazole are dissolved in a solvent such as acetonitrile. Once the coupling reaction is complete, typically 1-10 min., the excess reagent is washed from the silica with solvents such as tetrahydroffuran and acetonitrile. If desired, any unreacted 5'-hydroxyl groups can be blocked by treatment with acetic anhydride in 2,6-lutidine/tetrahydrofuran prior to the solvent washes, although this step is not essential to the synthesis. Next, the phosphite ester formed by this coupling procedure is converted to the phosphate ester by oxidation with iodine in a mixture of water, 2,6-lutidine, and tetrahydrofuran. The excess iodine solution is then removed from the silica by extraction with methanol and then with nitromethane to prepare for the next 5'-DMTr removal.

The above process is repeated with the desired nucleoside additions until the correct oligonucleotide sequence is obtained. Then, the methyl groups contained on the phosphotriesters are removed by treatment with thiophenol/triethylamine/dioxane (a 1/1/2 mixture by volume) for 45 min. at room temperature. This step is followed by treatment with concentrated ammonium hydroxide at room temperature for 3 hr. to hydrolyze the ester joining the 3' end of the oligonucleotide to the support, filtration of the support from the cleaved oligonucleotide, and warming of the filtrate at 50°C for 12 hr. Then, the oligonucleotide is purified by high-performance liquid chromatography, with the remaining 5'-DMTr group as a marker during the chromatography, and finally the 5'-DMTr group is removed by treatment with 80% acetic acid. Alternatively, the 5'-DMTr group can be removed prior to purification if the chromatographic method is replaced by polyacrylamide gel electrophoresis.

One of the main advantages of the Caruthers et al. process is its potential speed. High yields can be maintained throughout the process, thus extending the length of sequence that can be synthesized, and no intermediate purification steps are required. However, it is

highly labor intensive, and automation of the various steps in the process, particularly those involving the repetitive coupling of additional monomers to the growing chain, is desirable. Caruthers et al. have described a manually-operated apparatus in which the synthesis is performed, but this apparatus is not suitable for full automation because it contains no means of in situ formation of fully activiated nucleosides immediately prior to coupling. This is an important limitation because of the instability associated with handling these compounds. The present invention overcomes this limitation by incorporating a prereaction vessel in which the partially activated nucleosides are fully activated immediately prior to their introduction to the primary reaction vessel containing the solid support on which the oligonucleotide is synthesized.

Basically, while Caruthers et al employs a step-wise reaction process, the present invention differs markedly in providing a process wherein fully activated nucleosides are simultaneously reacted in a reaction zone comprising the primary reaction vessel containing the solid support.

This allows full automation of the process. Moreover, it permits use of much smaller amounts of expensive chemicals than are required with the manual activation technique. Also, it allows simple and reliable incorporation of any or all of the four nucleoside monomers at any coupling step in the synthesis process. This is not readily accomplished when using the manual activation process, and it is essential when a large set of DNA molecules with similar sequences must be made.

Another drawback to the Caruthers et al. apparatus is its use of a mechanical pump to move liquid through the reaction vessel containing the silica support. This mechanical system is subject to stalling of flow caused by formation of gas bubbles or precipitate in the pumping chambers. Also, the pumping action introduces pressure pulsations that can lead to fragmentation of the silica support and clogging of the reaction vessel and lines. The pump also introduces considerable dead volume into the lines leading into and out of the reaction vessel. This dead volume, along with the requirement of avoiding introduction of gas into the lines, leads to significant dilution of reagent concentrations or, alternatively, to use of much larger volumes of expensive reagents to fill the dead volume. The Caruthers et al. apparatus incorporates a recycle valve that allows the diluted reagents to be pumped again and again through the reactor,but this extends the reaction and solvent flushing times. The present invention uses pneumatic gas pressure in the reservoirs holding the reagents and solvents to effect the movement of these chemicals through the reactor. Moreover, it incorporates the use of miniaturized zero-dead-volume valves that minimize reagent consumption and allow coupling cycle times as low as 15 min. This mechanical system is not subject to blockage by gas bubbles (in fact it uses inert gas to purge most of the excess reagents from the lines and reactors prior to complete purging by solvents) and, since it does not use pulsing mechanical pumps, it does not produce physical destruction of the silica support.

SUMMARY OF THE INVENTION

Briefly, this invention comprises a novel apparatus for the synthesis of more complex oligonucleotide compounds from simpler chemical compounds wherein at least one of said simpler chemical compounds is carried on a solid matrix comprising a plurality of beads located within a reaction chamber and is sequentially subjected to a plurality of other simpler chemical compounds passed through the chamber in a stream, causing chemical interaction between the simpler chemical compounds to form said more complex compounds. Some of the simpler chemical compounds are mixed together in a prereaction vessel prior to their transfer through the chamber in order to generate the appropriate reactive species.

In another aspect, this invention comprises a novel process which comprises the introduction into a primary reaction zone containing therein a solid support, a plurality of fully activated nucleosides and permitting said nucleosides to react on said solid support to form an oligonucleoside.

It is an object of this invention to provide a novel apparatus for the synthesis of oligonucleotides.

More particularly, it is an object of this invention to provide an improved apparatus for the synthesis of oligonucleotides.

It is also an object of this invention to provide a novel method of synthesizing an oligonucleoside by the simultaneous reaction in a reaction zone of a plurality of interreactive and fully activated nucleosides.

These and other objects and advantages of this invention will be apparent from the detailed description which follows taken in conjunction with the accompanying drawings.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Turning to the drawings:

Figures 1A and 1B taken side-by-side depict, in schematic form, the novel apparatus of this invention.

Figure 2 is a sectional view of the primary reactor identified in Figure 1B.

Figure 3 is an enlarged partial section of the center portion of Figure 2.

Figure 4 is a sectional view of the prereaction vessel identified in Figure 1A.

Considering now the drawings in more de-

tail, the apparatus 10 of this invention is shown in Figures 1A and 1B.

Typically, solvent container 12 communicates with valve 14, pressure regulator 16 and valve block 18. Line 20 provided with pressure regulator 22 supplies an inert gas such as argon. The source of the inert gas is not shown in the drawings. Reservoir 24 preferably contains a simple chemical reactant such as tetrazole and is also associated with valve 26 and pressure regulator 28.

Reservoir 30 contains a single, protected, partially-activated nucleoside derivative such as that for adenosine. Similarly, reservoirs 32, 34, and 36 contain other nucleoside derivatives, such as those for thymine, guanine, and cytosine, respectively.

Reservoirs 30, 32, 34 and 36 are provided with valves 38, 42, 46, and 50,respectively, and with pressure regulators 40, 44, 48, and 52, respectively.

The container 12 and reservoirs 24, 30, 32, 34 and 36 have individual flow valves 54 and flow valve regulators 56 that allow the reservoirs to be vented to waste trap 114.

The valve block 18 feeds the prereactor 58 through line 404. The valve 60 and line 400 vent prereactor 58 to waste trap 114. Prereactor 58 communicates with valve block 62 by line 402. An inert gas is fed to valve block 62 by line 64. Line 66 vents to waste trap 114.

The admixture of simple nucleosides in prereacted form move to valve block 70 via line 72.

Reservoirs 80, 82, 84, 86 and 88 communicate with valve block 70.

Reservoirs 80, 82, 84, 86 and 88 each have appropriate valves 90, pressure regulators 92, flow valves 94, and flow valve regulators 96.

The primary reactor 98 is located within a thermostatted environment 100 and defines reaction chamber 102 having inlet 104 and outlet 106.

The outlet 106 connects to valve block 108.

The valve block 108 communicates with fraction collector 112. Valve block 108 also communicates with waste trap 114 either through line 198 or through flow regulator 200 and line 202.

Valve blocks 18, 62, 70 and 108 and valves 14, 26, 38, 42, 46, 50, 54, 60, 90, and 94 are electrically controlled by control unit 68.

The primary reactor apparatus 98 is shown in detail in Figures 2 and 3 to comprise a base 122 supporting a sleeve 124 which contains elements 126, 128, 130 and 132.

Below base 122 is the valve block 108, which has been discussed above in relation to Figures 1A and 1B.

The elements 122 and 124 are held together by internally-threaded collar 134.

Upper collar 136 keeps element 132 in sealing relationship within sleeve 124.

The chamber 102 is filled with macroporous beads comprising silica spheres having holes. The beads may also be chemically modified in other ways.

The prereactor 58 is show in detail in Figure 4 to comprise a vial 420, a screw cap enclosure 410, a series of connecting tubes 400, 402, and 404, and a sealing washer 440. Tubes 400, 402, and 404 communicate with valve blocks 60, 62, and 18, respectively. The nucleoside activation occurs in cavity 430 inside vial 420. The matching threaded surfaces 412 and 422 on 410 and 420, respectively, allow compression of washer 440 on surfaces 414 and 416 to seal the cavity 430 except to the connecting tubes.

Valve blocks 18, 62, 70, and 108 are multiunit valves preferably constructed of a zero-dead-volume configuration. Valve blocks such as those described by Wittmann et al. (U.S. Patent No. 4,008,736), Hunkapiller and Hood (U.S. Patent No. 4,252,769), Hood et al. (U.S. Patent Application No. 380,109), and Stark (U.S. Patent Application No. 300,184) are acceptable. The individual valve units in the blocks are electronically controlled on an individual basis by control unit 68.

TYPICAL OPERATION FOR SYNTHESIS OF OLIGONUCLEOTIDE

The use of the apparatus described above to synthesize an oligonucleotide consists of three basic procedures: loading the solid support containing the first linked nucleoside into the primary reactor, performing the coupling cycle described below one or more times using any or all nucleoside derivatives as desired at each cycle, and removal of the completed support-bound nucleotide from the primary reactor followed by cleavage from the support and removal of the protecting groups from the nucleotide. The order of the nucleoside additions is programmed into the control unit 68 prior to commencing the coupling cycle operation.

In order to load the support containing the first linked nucleoside into the chamber 102 of primary reactor 98, the primary reactor is partially disassembled by loosening the tubing retaining screw 103 and removing, in order, upper collar 136, locking ring 121, Teflon washer 123, element 132, porous Teflon washer 127, element 130, and, together, element 128, porous Teflon washer 125, and element 126. Any previously used support material is emptied from chamber 102 and the porous Teflon washers 125 and 127 are discarded. The primary reactor 98 is then reassembled by replacing, in order, element 126, a new porous Teflon washer 125, element 128, element 130, new support containing the attached first nucleoside into

chamber 102, porous Teflon support 127, element 132, Teflon washer 123, locking ring 121, and upper collar 136. Assembly is completed by tightening tubing retaining screw 103.

A typical coupling cycle consists of the following steps: Step 1. Duration: 30 seconds. Flush nitromethane (2.5 ml) through primary reactor. Effluent is directed through line 198.

Step 2. Duration: 10 seconds. Flush argon through primary reactor to remove nitromethane. Effluent is directed through line 198.

Step 3. Duration: 30 seconds. Flush 3% trichloroacetic acid in chloroform (1.5 ml) through primary reactor to effect detritylation. Effluent is directed into fraction collector 112.

Step 4. Duration: 10 seconds. Flush argon through primary reactor to remove acid solution. Effluent is directed into fraction collector 112.

Step 5. Duration: 20 seconds. Flush nitromethane (1.5 ml) through primary reactor. Effluent is directed into fraction collector 112.

Step 6. Duration: 10 seconds. Flush argon through primary reactor to remove nitromethane. Effluent is directed into fraction collector 112.

Step 7. Duration: 20 seconds. Flush methanol (1.3 ml) through primary reactor. Effluent is directed through line 198.

Step 8. Duration: 10 seconds. Flush argon through primary reactor to remove methanol. Effluent is directed through line 198.

Step 9. Duration: 30 seconds. Pressurize nucleoside reservoirs with argon.

Step 10. Duration: 15 seconds. Flush acetonitrile (1.1 ml) through primary reactor. Effluent from primary reactor is directed through line 198. Deliver nucleoside phosphoramidite in acetonitrile (0.2 ml) to prereactor.

Step 11. Duration: 5 seconds. Flush acetonitrile (0.4 ml) through primary reactor. Effluent from primary reactor is directed through line 198. Flush argon through prereactor to push nucleoside phosphoramidite into prereactor.

Step 12. Duration: 30 seconds. Pressurize tetrazole reservoir.

Step 13. Duration: 20 seconds. Flush acetonitrile (1.4 ml) through primary reactor. Effluent from primary reactor is directed through line 198. Deliver tetrazole in acetonitrile (0.4 ml) to prereactor.

Step 14. Duration: 5 seconds. Flush acetonitrile (0.4 ml) through primary reactor. Effluent from primary reactor is directed through line 198. Flush argon through prereactor to push tetrazole into prereactor.

Step 15. Duration: 15 seconds. Flush acetonitrile (1.1 ml) through primary reactor. Effluent from primary reactor is directed through line 198. Bubble argon into prereactor to mix nucleoside and tetrazole solutions and effect activation of nucleoside for coupling.

Step 16. Duration: 10 seconds. Flush argon through primary reactor to remove acetonitrile. Effluent is directed through line 198.

Step 17. Duration: 2 seconds. Pressurize prereactor with argon.

Step 18. Duration: 360 seconds. Push activated nucleoside from prereactor through primary reactor with argon directed into prereactor. This process effects coupling of the activated nucleoside to the nucleotide on the support in the primary reactor. Effluent from primary reactor is directed through flow regulator 200.

Step 19. Duration: 5 seconds. Flush argon through prereactor and primary reactor to remove activated nucleoside. Effluent from primary reactor is directed through line 198.

Step 20. Duration: 10 seconds. Deliver acetonitrile (1.5 ml) to prereactor.

Step 21. Duration: 30 seconds. Push acetonitrile from prereactor through primary reactor with argon directed into prereactor. Effluent from primary reactor is directed through line 198.

Step 22. Duration: 10 seconds. Flush acetonitrile (0.7 ml) through primary reactor. Effluent from primary reactor is directed through line 198. Deliver acetonitrile (1.5 ml) to prereactor.

Step 23. Duration: 25 seconds. Flush acetonitrile (1.8 ml) through primary reactor. Effluent from primary reactor is directed through line 198. Flush argon through prereactor to remove acetonitrile. Effluent from prereactor is directed through line 66.

Step 24. Duration: 10 seconds. Flush argon through primary reactor. Effluent is directed through line 198.

Step 25. Duration: 60 seconds. Flush iodine/water/ 2,6-lutidine/tetrahydrofuran solution (2.0 ml) through primary reactor to effect oxidation from phosphite to phosphate. Effluent is directed through line 198.

Step 26. Duration: 10 seconds. Flush argon through primary reactor to remove iodine solution. Effluent is directed through line 198.

Step 27. Duration: 60 seconds. Flush methanol (3.0 ml) through primary reactor. Effluent is directed through line 198 Step 28. Duration: 10 seconds. Flush argon through primary reactor. Effluent is directed through line 198.

Once the coupling cycle described above is repeated the desired number of times, the completed support-bound oligonucleotide is removed from the primary reactor in order to provide cleavage of the support and base-protecting groups. Removal from the primary reactor is accomplished by repeating the procedure described above for loading new silica support into the primary reactor. The cleavage and deblocking process is then performed manually outside the apparatus 10 by a method such as that described by Caruthers et

al.

Variations in the standard coupling cycle can include the use of $ZnBr_2$ (saturated solution in nitromethane) rather than the trichloroacetic acid solution for the detritylation step. In this case, the detritylation must be extended to 10 to 20 min. Another variation is to deliver more than one activated nucleoside to the primary reactor during the coupling step. This is accomplished by delivering two, three, or four partially activated nucleosides to the prereactor (using 1/2, 1/3, or 1/4 the normal one nucleoside delivery time, respectively), adding the tetrazole to the nucleoside mixture, and forcing the mixture of activated nucleosides through the primary reactor.

The completeness of the coupling reactions can be monitored conveniently by collecting, at each cycle, the effluent from the detritylation and subsequent solvent flush steps and measuring the absorbance at 498 nm of the dimethoxytrityl cation in a spectrophotometer. The relative amounts of absorbance present in each succeeding tube of the fraction collector can be used to calculate a cycle coupling yield. Typical yields are 95% or greater.

CLAIMS

1. Apparatus for use in the synthesis of more complex oligonucleotide compounds from simpler chemical compounds, which apparatus comprises a solid matrix comprising a plurality of beads for carrying at least one of said simpler chemical compounds and located within a reaction chamber, means for sequentially passing a plurality of other simpler chemical compounds through the reaction chamber in a stream to cause chemical interaction between the simpler chemical compounds to form said more complex compounds, and a pre-reactor in fluid communication with said reaction chamber wherein partially activated nucleosides can be fully activated immediately prior to their introduction as said other simpler chemical compounds into said reaction chamber.

2. Apparatus according to claim 1 wherein a series of reservoirs for holding reagents and solvents are provided upstream of said reaction chamber and in fluid communication therewith, and pneumatic gas pressure means are provided in association with said reservoirs to efect pneumatic movement of said reagents and solvents through said reaction chamber.

3. Apparatus according to claim 1 or 2 wherein a series of reservoirs for holding reagents and solvents are provided upstream of said pre-reactor and in fluid communication therewith.

4. Apparatus according to claim 1, 2 or 3 wherein valve blocks are provided upstream of said pre-reactor and between said pre-reactor and said reaction chamber to control fluid flow, said valve blocks being of zero-dead-volume.

5. Apparatus according to any one of the preceding claims wherein said beads comprise macroporous silica spheres containing holes therein.

6. Apparatus according to any one of the preceding claims wherein a valve block is also provided downstream of said reaction chamber and in fluid communication therewith, said valve block further communicating with a fraction collector.

7. Apparatus according to any one of the preceding claims wherein said beads are supported by a porous polytetrafluoroethylene sheet forming the bottom of said reaction chamber.

8. Apparatus according to claim 1 substantially as described with reference to the accompanying drawings.

9. A process which comprises the introduction into a primary reaction zone containing therein a solid support of a plurality of fully activated nucleosides and permitting said plurality of nucleosides to react simultaneously on said support to form an oligonucleoside, wherein partially activated nucleosides are fully activated in a pre-reaction zone just prior to introduction into the primary reaction zone.

10. A process according to claim 9 wherein the solid support carries a nucleotide and said plurality of activated nucleosides couple to said nucleotide.

11. A process for the synthesis of more complex oligonucleotide compounds from simpler chemical compounds wherein at least one nucleotide as a simpler chemical compound is carried on a solid matrix comprising a plurality of beads located within a reaction chamber and is sequentially subjected to a plurality of other simpler chemical compounds passed through the chamber in a stream, causing chemical interaction between the simpler chemical compounds to form said more complex compounds, and wherein partially activated nucleosides are fully activated immediately prior to their introduction as said other simpler chemical compounds into said reaction chamber.

12. A process according to claim 11 wherein the partially activated nucleosides are fully activated in a pre-reactor which is in fluid communication with the reaction chamber.

13. A process according to claim 11 or 12 wherein reagents and solvents are held in a series of reservoirs provided upstream of said reaction chamber and in fluid communication therewith, and these materials are moved pneumatically through said reaction chamber by means of pneumatic gas pressure means provided in association with said reservoirs.

14. A process according to claim 11, 12 or 13 wherein said beads comprise macroporous silica spheres containing holes therein.

15. A process according to claim 14 wherein said macroporous silica spheres are supported by a porous polytetrafluoroethylene

sheet forming the bottom of said reaction chamber.

16. A process according to any one of claims 11 to 15 wherein reagents and solvents are also held in a series of reservoirs provided upstream of said pre-reactor and in fluid communication therewith.

17. A process according to any one of claims 11 to 16 wherein fluid flow upstream of said pre-reactor and between said pre-reactor and said reaction chamber is controlled by valve blocks of zero-dead-volume.

18. A process according to any one of claims 11 to 17 wherein fluid flow downstream of said reaction chamber is controlled by a valve block further communicating with a fraction collector.

19. A process according to claim 11 substantially as described by reference to Figs. 1A and 1B of the accompanying drawings.

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